

UNIVERSITE DE GENEVE  
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Clinique universitaire d'ophtalmologie

FACULTE DES SCIENCES  
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AN ULTRASTRUCTURAL AND CYTOCHEMICAL STUDY OF  
DIFFERENT FUNCTIONAL STATES OF THE ALBINO  
RAT'S RETINAL PIGMENT EPITHELIUM

THESE

présentée à la Faculté des sciences de l'Université de Genève  
pour obtenir le grade de docteur ès sciences biologiques

par

Marie-Louise VON SCHACK-BEAUCHEMIN

du

CANADA

Thèse No 1808

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La Faculté des sciences, sur le préavis de Messieurs H. Huggel, professeur ordinaire et P. Leuenberger, privat-docent (Faculté de médecine — Genève), codirecteurs et J.W. Rohen, professeur (Université d'Erlangen — Nürnberg), autorise l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

Genève, le 22 mars 1977

*Le doyen :*  
Jean Muller

Thèse No 1808





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## I N T R O D U C T I O N

The retinal pigment epithelium (RPE) has long been recognized for its vital role played in the physiology of vision (Kühne, 1879), however its functional activities in relation to the surrounding choriocapillaris and sensory retina have only recently been the subject of more detailed research.

From the investigations of Bairati and Orzalesi (1963), Young and Droz (1968), and Young and Bok (1969), it was first suggested and then confirmed that one of the principal functions of the RPE is that of phagocytosis of the shedded outer portion of the rod outer segments (ROS). Since ROS discs have been shown, in the frog retina, to be made up of 60 % protein, the majority of which is opsin (Bibb and Young, 1974), it was soon realized that phagocytosis must play an important role in the rhodopsin turn-over.

The RPE cells have an extremely low rate of mitosis (Mann, 1928) and therefore must bear the burden of the continually ingested material. As such it was postulated that the RPE cell must have an extremely active phagolysosomal system (Feeney, 1973).

One of the main purpose of this thesis was to study some morphological aspects of this phagolysosomal system in the albino rat's RPE at different developmental stages as well as under experimental conditions. Parallel to all ultra-structural studies carried out, cytochemical procedures were used to detect sites of acid phosphatase activity (acPase) and glucosaminidase activity in the RPE, two well known hydrolytic enzymes (Tappel, 1969). Until now, relatively few cytochemical studies have been carried out on the RPE,

with a few exceptions (Abraham et al., 1969; Ansell and Marshall, 1974; Ishikawa and Yamada, 1970; Marshall, 1970; Marshall and Ansell, 1971).

The adult albino rat has no melanin in its cells because of a genetical block in tyrosinase activity (Moyer, 1969). In the embryonic and post natal RPE of the albino rat, however, premelanosomes are present and we were interested to study not only their normal development but also their subsequent disappearance as well as part of their enzymatic activities.

In senile animals, emphasis was put on studying the formation and accumulation of lipofuscin granules resulting especially from degraded phagosomes which develop in great quantity in the RPE.

One of the experimental conditions used in this study was the rearing of young animals in darkness in order to see if there would be any light effect firstly on the development and disappearance of premelanosomes and secondly on the cytogenesis of the phagosomes.

A second experimental condition used was the administration of colchicine, a known inhibitor of normal microtubular function (Borisy and Taylor, 1967a, 1967b; Shelanski and Taylor, 1967, 1968). In using this blocking agent, we hoped to get a new insight into phagosome-lysosome interaction and into intracellular transport in the RPE.

## M A T E R I A L   A N D   M E T H O D S

The animal used for this study was the albino rat. With the exception of the studies of mature (40 weeks old) and senile (120 weeks old) rats, which were of a Wistar strain, the SIV-50 rat, being of a Sprague-Dawley strain, was used throughout this study.

### A. Normal Conditions

Rats were subjected to normal cyclic light and their eyes enucleated under ether anaesthesia. The different developmental and functional stages of the RPE were studied in rats having the following ages : 16 to 19 days of gestation and 20 to 36 hours after birth : 1, 2, 3, 4, 8, 24, 40 and 120 weeks old. All specimens were prepared for electron microscopy and for cytochemical studies.

### B. Experimental Conditions

#### 1. Dark Rearing

Pregnant female rats were kept in a totally dark room of 20° C. The new born rats were then sacrificed, 2 at a time, when they had reached the age of 1, 2, 3 and 4 weeks. A red 25 Watt photographic light bulb located on the ceiling served as illumination during the enucleation of the young rat's eyes.

#### 2. Colchicine Administration

Young rats weighing 100 to 150 grams, received an intravenous injection of a 0.5 ml solution of 0.5 to 1.0 mg colchicine per 100 gm body weight. Control animals received a 0.5 ml solution of 0.9 % NaCl. An animal of each group was successively sacrificed 2½, 4 and 8 hours after injection.

### C. Conventional Electron Microscopy

Small slices of the retina were fixed for 90 minutes to 2 hours in a solution of 3 to 4 % glutaraldehyde in a 0.1 M phosphate buffer of pH 7.4; 5 % sucrose was added to the buffer solutions. The tissue slices were rinsed in a 0.1 M phosphate buffer and post-fixed in 1 %  $\text{OsO}_4$  for  $1\frac{1}{2}$  hours. The tissue was then block stained in 0.5 % uranyl acetate, pH 5, for 1 to 2 hours at room temperature. After dehydration in a graded series of alcohol followed by propylene oxide, the tissue was embedded in Epon-812.

Thin sections of 300 to 500 Å were cut with a diamond knife on a Porter-Blum microtome and collected on bare copper grids. They were then stained for 2 minutes with lead citrate and examined with a Philips 300 electron microscope at 80 Kv using 30 µm objective apertures.

### D. Cytochemistry

#### 1. Optical Microscopy

Freshly enucleated posterior eye cups were fixed for 60 to 90 minutes in 3 % glutaraldehyde buffered in 0.1 M sodium cacodylate with a pH of 7.4. The tissue was then rinsed in the cacodylate buffer for 2 to 6 hours after which it was cut 10 to 20 µ in thickness on a Leitz freezing microtome.

##### a) Lead Precipitation Method

Frozen sections were placed in a modified Gömöri medium, containing the substrate cytidine monophosphate (CMP) for 20, 40 and 60 minutes at 37° C, after which they were rinsed in distilled water, visualised in ammonium sulphide and mounted with glycerol on glass slides.

##### b) Azo Dye Method using Pararosanilin

Frozen sections were incubated in a pararosanilin solution containing the substrate N-acetyl-β-D glucosamine Naphtyl AS-LC for 90 minutes. They were then dehydrated in a graded

series of alcohol followed by xylol and mounted with Permount on glass slides.

## 2. Electron Microscopy

Tissue was fixed in a similar manner to that used for optical microscopy except that it was cut into slices approximately 0.25 mm in thickness. The tissue was then rinsed for 2 to 6 hours in 0.1 M sodium cacodylate buffer with 5 % sucrose, followed by a 20 minutes incubation at 37° C in the modified Gömöri medium containing CMP. It was then rinsed in 0.1 M cacodylate buffer with 5 % sucrose and post-fixed in 1 % OsO<sub>4</sub> for 1½ hours. The procedures following this are the same as those mentioned in "C".

Control cytochemical preparations were carried out firstly with an incubation of the tissue in the medium without the substrate, and secondly by preheating the tissue to 80° C for 20 minutes before being incubated in the medium.



## R E S U L T S

### I. General Description

The RPE of the SIV-50 rat is an inconspicuous single layer of cells located between the choriocapillaris and the sensory retina, the former of which shows thin fenestrated blood vessels facing the RPE.

The rat has an all rod retina whose outer segments are long rod like structures with flattened discs stacked in rows, similar to those observed in other vertebrates (Cohen, 1960, 1961, 1964; Nir & Pease, 1973). These outer segments are intimately surrounded by apical cytoplasmic projections or microvilli of the RPE (diagram 1 d-f). The basal or chorioidal part of the RPE is seen to have infoldings in the mature animal. Mitochondria are frequently found in a basal position.

The cytoplasm is rich in cell organelles, notably endoplasmic reticulum (ER) and in mature and older animals, residual bodies (diagram 1 e-f). Lipid droplets are regularly found in the basal part of the cytoplasm. In younger animals premelanosomes are found in the apical cytoplasm.

### II. Detailed Description of the Developmental Stages of the RPE

#### A. 16 to 19 days of gestation

Well developed rough ER (RER) cisternae, some being exceptionally long, are particularly evident at this stage (diagram 1a) along with abundant free ribosomes. Smooth ER (SER), however, is less noticeable in the cytoplasm.

The basally oriented plasma membrane starts to show infoldings whereas apically it extends fingerlike projections.

### Diagram 1

A schematic summary of the different developmental stages of the SIV-50 albino rat's RPE. Sites of acPase activity are indicated with an asterix.

#### a) Embryo :

Long parallel SER tubules as well as premelanosomes (P) in different stages of development are very abundant in the cytoplasm at this stage. The Golgi apparatus (G) consists only of closely associated tubules and cisternae with occasional coated vesicles (CV). Mitochondria (M) are small with immature cristae. The basally located plasma membrane (BI) shows only slight infoldings and the apical plasma membrane (MV) can be seen to have small projections. Dense bodies (DB) can be seen often appearing to develop from smooth endoplasmic reticulum (SER).

#### b) Post-natal :

The randomly scattered RER tubules have become shorter and premelanosomes (P), in different developmental stages, are slightly more numerous at this stage. The Golgi apparatus (G) consists of a more denser arrangement of tubules and cisternae with closely associated SER tubules. Mitochondria (M) show slightly more developed cristae. The apical microvilli (MV) have become longer and appear to grow along side the developing rod inner segments (RIS).

#### c) 1 week :

More SER tubules can be seen at this stage and the RER tubules have become shorter. Premelanosomes (P) are wider with a more loosely arranged matrix; dense bodies (DB) can often be seen in their vicinity. The Golgi apparatus (G) is mature with a well developed GERL (GL). Mitochondria (M) are more developed and are located mainly in the basal cytoplasm. The apical microvilli (MV) are longer and thinner and can be seen near the growing rod outer segments (ROS).

d) 2-3 weeks :

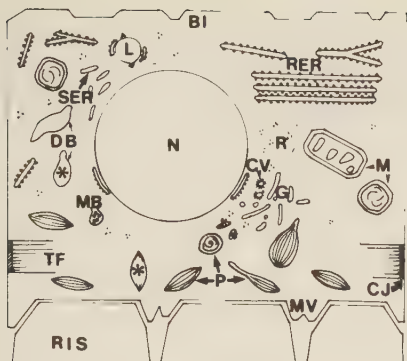
The cytoplasm is especially rich in SER. Premelanosomes (P) have become scarce and those which can be seen have associated dense bodies (DB), the latter being more numerous in the cytoplasm and often found near phagosomes (PH) now present in the cytoplasm. Mature mitochondria (M) are mostly basally oriented. The long, slender microbilli (MV) can be seen surrounding and sometimes engulfing the outermost part of the ROS.

e) 40 weeks :

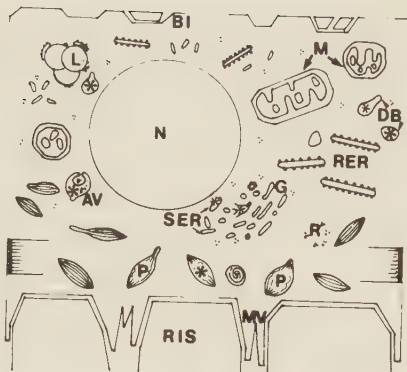
Many phagosomes (PH) often being transformed into apically located residual bodies (RB) can be seen at this stage. Dense bodies (DB) can often be found in their vicinity, if not in direct contact with them. The basal infoldings (BI) are well indented, showing a marked increase in surface area. Premelanosomes are completely absent at this stage.

f) 120 weeks :

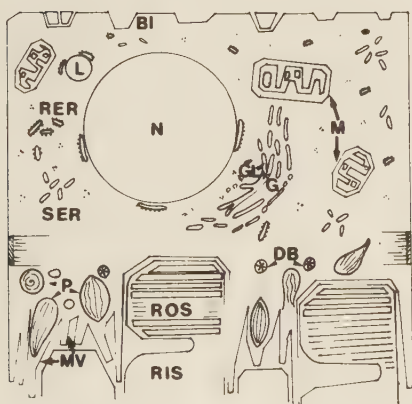
Ingested phagosomes (PH) and resulting residual bodies (RB), both showing acPase activity are particularly evident at this stage. Some of them can be seen as lipofuscin granules (LF) containing a dark fuscine pigment as well as lipid droplets (L) in a granular matrix. They are mostly apically located. A mitochondrion (M) being autophagocytised can be seen. ER profiles have decreased somewhat and acPase-positive dense bodies (DB) have become very abundant in the cytoplasm, the latter being found especially near to or in direct contact with the phagosomes or residual bodies.



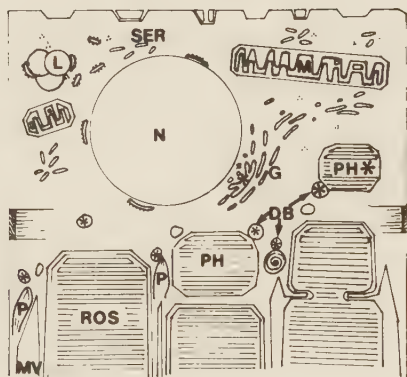
a



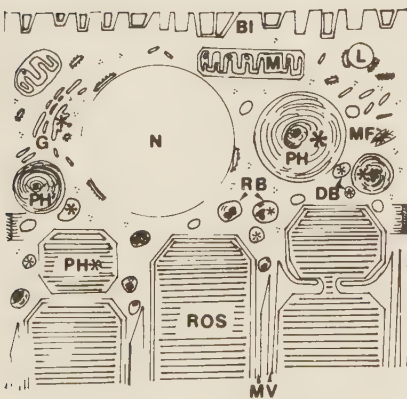
b



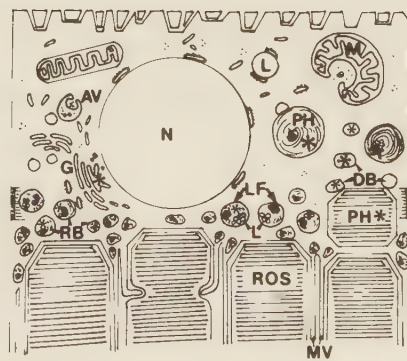
c



d



e



f

Premelanosomes are abundantly seen in the apical cytoplasm and show a matrix containing fine parallel filaments (diagram 1a, 2d) with a periodicity. Their form may be elongated or of a slightly wider, cigar-shape. The Golgi apparatus can be identified as an aggregation of tubules and vesicles (diagram 2a). Mitochondria are rather small with immature cristae (diagram 2c) and can be found scattered at random throughout the cytoplasm. Other cell organelles readily identified in the embryonic tissue are lipid droplets, multivesicular bodies, autophagic vacuoles and dense bodies.

Acid phosphatase activity was most prominent on the premelanosomes. Dense bodies, autophagic vacuoles and SER tubules lying close to the Golgi apparatus, as well as a few isolated cytoplasmic SER tubules likewise displayed acPase activity.

#### B. 20 to 36 hours after birth

Most of the RER cisternae have become shorter (diagram 1b) and a slight increase in SER can already be observed in the cytoplasm. The Golgi apparatus has developed into a network of tubules and vesicles organized into layers with associated SER tubules (diagram 2a).

Apically oriented premelanosomes can be seen to have the same forms as those seen in embryological tissue as well as larger more ellipsoid forms with a more loosely arranged matrix (diagram 1b). The apical microvilli have become longer and thinner. Mitochondria are larger with more developed cristae (diagram 1b, 2c). Autophagic vacuoles are quite abundant.

At a light microscopic level, acPase activity can be seen to be uniformly distributed throughout the cell; at an electron microscopic level, it is preferentially localized



## Diagram 2

A schematic summary of the early development of various cell organelles in the RPE of the SIV-50 albino rat.

### a) Golgi apparatus :

Initially seen to be composed of a few scarce cisternae and vesicles, the apparatus then grows in a regular manner with the cisternae becoming longer and more parallel and the vesicles orienting themselves on one, slightly concave, side of the cisternae. When mature at 1 week of age, it can be seen to be composed of a large network of parallel cisternae, associated tubules and coated vesicles (CV) and a well developed GERL (GL).

### b) Endoplasmic reticulum :

Long parallel RER profiles are particularly evident in the embryonic stages, along with shorter, but still somewhat well developed RER profiles. The very long profiles become shorter in the post natal stages and a few SER can be found scattered among the RER-heavily laden cytoplasm. As development continues, the RER profiles become even shorter and the SER tubules increase accordingly in number. By 2 to 4 weeks, SER profiles have become very abundant and already outnumber the RER whose profiles are about as large as those of the SER.

### c) Mitochondria :

In the early stages, the mitochondria appear as small organelles with poorly defined cristae. As the organelle develops, the cristae show more evident indentations and the organelles as a whole becomes larger. When mature, it can be very long in size with numerous highly developed cristae.

d) Premelanosomes :

I. This is the initial stage in which the premelanosome can be seen developing from SER profiles. One end of the organelle starts to widen whereby the organelles takes on a cigar-shape. In this early stage, a fine filamentous matrix with a periodicity can already be seen.

II. This is the mature premelanosomal stage, the whole organelle having taken on a cigar-shape with a dense matrix containing a tightly coiled filamentous structure. When cut transversally, the filamentous structure can be seen to be somewhat spiral in arrangement.

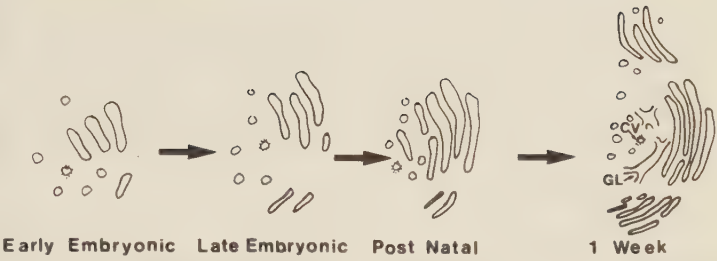
III. Since no melanization takes place due to the lack of tyrosinase, the organelle begins, at this stage, to break-down. It becomes wider and the matrix more loosely arranged. Occasional dense bodies (DB) can be found in their vicinity.

Some of the widened granules, with a loosely arranged matrix can often be seen to have apparent open ends, or an unclearly defined membrane delimitation. Very often, dense bodies are seen near the organelles at this stage.

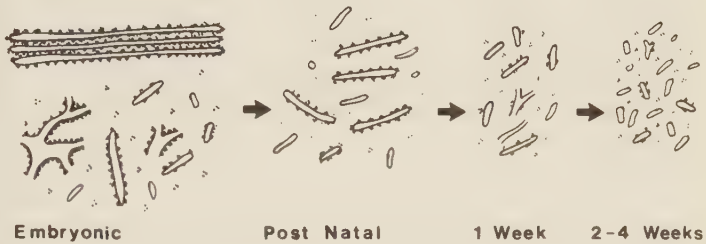
IV. Dense bodies (DB) can now be seen to be in direct contact with the enlarged organelles whose matrix has become very closely arranged at this stage, often with clumpings seen on the loosened filamentous strands.

V. At this stage, lysosomal digestion of the premelanosomes has proceeded to a stage whereby the organelle has decreased drastically in size and the contents greatly condensed. In the final stage, the organelle can be recognized as a residual body with a densely staining matrix often with lamellar-like inclusions.

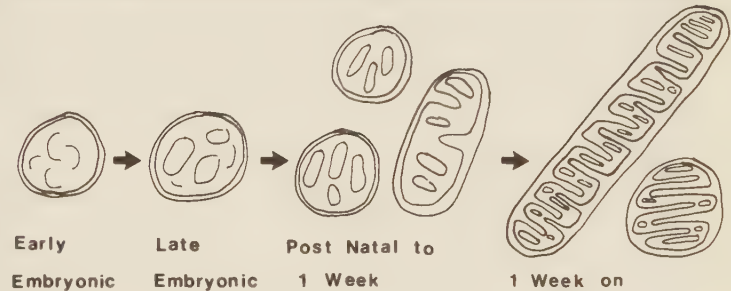
# Golgi Apparatus



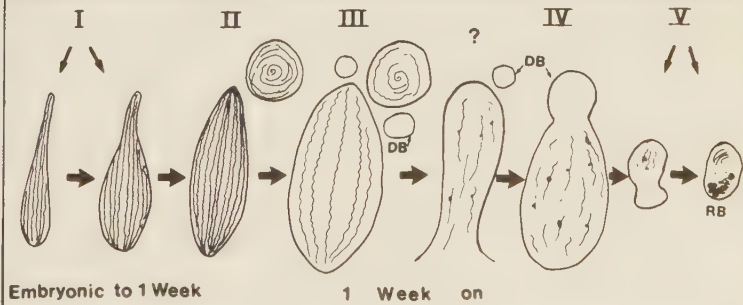
# Endoplasmic Reticulum



# Mitochondria



# Premelanosomes



on the premelanosomes. Dense bodies, Golgi-oriented SER tubules and autophagic vacuoles also readily display acPase activity (diagram 1b).

C. One week old

1. Animals subjected to normal cyclic light

The apical microvilli have become somewhat longer and thinner and are in intimate contact with the developing rod outer segments (diagram 1c). The cytoplasm is rich both in SER and RER. The Golgi apparatus is mature at this stage and consists of 3 to 4 elements, the outer being somewhat fenestrated and the inner being more tubular, probably corresponding to GERL (Novikoff, 1964) (Diagram 1c, 2a). Premelanosomes are less numerous and mainly of a more ellipsoid shape with a loosely arranged matrix. Dense bodies are often seen in their vicinity. This may be considered an early degenerative stage (diagram 2d). Mitochondria are more numerous and larger with well developed cristae and are situated mainly in the basal cytoplasm.

2. Dark reared animals

Premelanosomes, especially in the early developmental stages seen in embryonic and post natal tissue are notably more numerous in the cytoplasm (table II). Diagram 2d schematically depicts the different developmental stages of the premelanosome. When the granule is transversally cut, the filamentous matrix is seen to have a spiral arrangement. Little acPase is seen on the premelanosome, unless in direct contact with acPase-positive dense bodies. AcPase activity can sometimes be seen on Golgi-associated SER tubules, or GERL, as well as on other SER tubules.

D. Two to three weeks old

1. Animals subjected to normal cyclic light

Phagosomes are present by 2 weeks and numerous by 3

weeks in the RPE cytoplasm, often with associated dense bodies. Apical microvilli have reached their maximum length and can often be seen engulfing the outermost tips of the rod outer segments (diagram 1d). A few rare premelanosomes can still be found in the apical part of the cytoplasm and are often in close association, or in direct contact with dense bodies. The cytoplasm is particularly rich in SER.

AcPase activity can be seen to be displayed on the phagosomes, dense bodies and occasionally on Golgi-oriented SER (GERL) tubules. Premelanosomes rarely show any more enzymatic activity.

## 2. Dark reared animals

By 2 weeks of age, no phagosomes were found in the RPE cytoplasm and by 3 weeks were only very rarely seen (table I). The rod outer segments had a slightly abnormal appearance and were seen to accumulate exterior to the apical microvilli. Premelanosomes were more numerous in the cytoplasm than in the controls (table II) and could be found in all the developmental stages (diagram 2d). Only very occasional premelanosomes, however, displayed acPase activity. Mitochondria appeared to be large and more numerous than in the control cell.

AcPase activity was particularly evident on the cytoplasmic SER tubules, as well as on the dense bodies.

## E. Four weeks old

### 1. Animals subjected to normal cyclic light

Numerous acPase-positive phagosomes in different stages of degeneration are observed in the RPE cytoplasm, often with one or more acPase dense bodies adjacent to them. The apical microvilli appear to be actively engaged in the pinching off of the outer most discs of the rod outer segments. A few rare

Table I and II

The average number of phagosomes (table I) and preme-lanosomes (table II) in the SIV-50 albino rat's RPE was counted after having converted the different micrographs to the same magnification. Compared are newborn animals, either exposed to cyclic light (normal or dark-reared, on an average, each micrograph contained 1 to  $1\frac{1}{2}$  RPE cells (n = number of micrographs counted)).

- I. At two weeks of age, when phagocytosis was first observed, phagosomes were very scarce in the control animals, however by three weeks of age, their number had greatly increased, whereas that of the dark-reared cells remained minimal. By four weeks of age, the average number of phagosomes in the control animals increased significantly, whereas that of the dark reared animals remained unchanged, staying at a minimal level. At 2 weeks 40 x more numerous in rats exposed to normal cyclic light.
- II. The RPE appeared to contain a maximum amount of premela-nosomes by one week of age, after which their average number progressively decreased until by four weeks of age only scarce granules were observed. Those counted in the dark reared animals remained significantly more numerous throughout the developmental stages shown and by four weeks of age a significant number of the preme-lanosomes could still be counted in the RPE cell.



T A B L E I

Ratio of phagosomes per micrograph

| Two weeks old  |                 | Three weeks old |               | Four weeks old |                |
|----------------|-----------------|-----------------|---------------|----------------|----------------|
| Cyclic light   | Dark reared     | Cyclic light    | Dark reared   | Cyclic light   | Dark reared    |
| 0.05<br>(n=18) | <0.01<br>(n=15) | 2.78<br>(n=9)   | 0.25<br>(n=4) | 3.5<br>(n=4)   | 0.09<br>(n=11) |
| ~ 5 x          |                 | ~ 10 x          |               | ~ 40 x         |                |

T A B L E I I

Average number of premelanosomes per micrograph

| One week old   |               | Two weeks old  |              | Three weeks old |               | Four weeks old |              |
|----------------|---------------|----------------|--------------|-----------------|---------------|----------------|--------------|
| Cyclic light   | Dark reared   | Cyclic light   | Dark reared  | Cyclic light    | Dark reared   | Cyclic light   | Dark reared  |
| 11.3<br>(n=11) | 23.4<br>(n=7) | 10.7<br>(n=13) | 12<br>(n=12) | 3.5<br>(n=10)   | 10.6<br>(n=5) | 1<br>(n=3)     | 6.5<br>(n=7) |

acPase-negative premelanosomes can still be found in the apical region of a few cells.

At a light microscopic level, glucosaminidase activity was clearly displayed on the RPE layer whereas barely any activity was seen on the choriocapillaris and on the sensory retina.

## 2. Dark reared animals

Phagosomes were practically never seen in the cytoplasm of the RPE cells studied (table I) even through the rod outer segments were in intimate contact with the proliferous apical microvilli. Large, loosely arranged premelanosomes showing barely any acPase activity, were numerous in some cells and quite are in others (table II). Mitochondria also appeared more numerous when compared to the average count of normal cells.

AcPase activity was abundant on the cytoplasmic SER tubules as well as on those closely associated with the Golgi apparatus (GERL) and on the dense bodies.

## F. 40 weeks old

Phagosomes, dense bodies and residual bodies, all showing acPase activity, were particularly abundant in the cytoplasm (diagram 1e). In some of the apically oriented residual bodies, a fuscine-like pigment could be seen along with small lipid droplets, in which case they were referred to as lipofuscin bodies (Frank and Christensen, 1968; Feeney et al., 1965). The cytoplasm showed a slight increase in microfibrillar content. The Golgi-oriented SER tubules sometimes displayed acPase activity.

At a light microscopic level, both acPase and glucosaminidase activity were regularly displayed on the RPE but were absent, or nearly so, on the surrounding tissue.

G. 120 weeks old

AcPase positive residual bodies, many of which were lipofuscin bodies and were located mainly in the apical part of the cytoplasm were overwhelmingly abundant in the RPE (diagram 1f). Many autophagic vacuoles were seen, often containing degenerating mitochondria. The cytoplasm appeared to show a net decrease in ER profiles.

AcPase activity, which varies considerably from cell to cell in ultrathin sections, but is more regularly dispersed in frozen sections, can often be seen in excess on the cytoplasmic SER tubules, on the apical microvilli and on the basal infoldings. Phagosomes, dense and residual bodies regularly displayed acPase activity.

III. The Effects of Colchicine on the RPE

A. Two and one-half hours after treatment

Dense bodies were seen to accumulate in the cytoplasm, especially near the Golgi region, along with associated autophagic vacuoles. In the control cells they were more dispersed throughout the cytoplasm. Membrane-bound crystalloid structures showing a periodicity, as well as microtubules and centrioles could occasionally be seen in the colchicine treated RPE cells. The morphology and localization of the phagosomes and mitochondria did not differ noticeably from the control cells.

At a light microscopic level, glucosaminidase activity, which was readily displayed on the RPE of both the colchicine treated and the control cells, was seen to be particularly clumped in the former and more evenly dispersed in the latter.

B. Four hours after treatment

Microtubules could no longer be identified in the colchicine treated cells, whereas they were regularly seen in the controls cells (diagram 3a). The Golgi-oriented dense body aggregations have become more obvious in the colchicine treated cell (diagram 3b). AcPase activity of these aggregations, although evident at an electron microscopic level, is particularly striking at a light microscopic level where it is seen clumped together on the RPE cell layer (diagram 3d). The control cells showed a more evenly dispersed enzyme activity on the RPE cell layer (diagram 3c). This same activity was also seen in tissue incubated for glucosaminidase activity. Occasional dense bodies could sometimes be seen near the acPase-positive phagosomes, the latter of which appeared to be normal in appearance in colchicine treated cells. Membrane-bound crystalloid structures were occasionally seen. Autophagic vacuoles were particularly evident in the colchicine treated cell (diagram 3b).

C. Eight hours after treatment

The dense body aggregations have become very conspicuous in the colchicine treated cell and readily display acPase activity at both an electron microscopic and light microscopic level. In frozen sections, they appear as a clumping of acPase positive granules. In some colchicine treated cells, a noticeable SER increase could be observed. No cytoplasmic microtubules were ever identified in the cytoplasm. Centrioles, as well as large lipid droplet accumulations were occasionally seen in the RPE after colchicine treatment. Phagosomes, in both control and colchicine treated cells, displayed acPase activity. In control cells, however, they were consistently surrounded by acPase-positive dense bodies.

Diagram 3

Semi-schematic diagram comparing the effects of colchicine administration on the SIV-50 albino rat's RPE cell to a control cell. The RPE can be seen at an electron microscopic magnification in a and b, and at a light microscopic magnification in c and d.

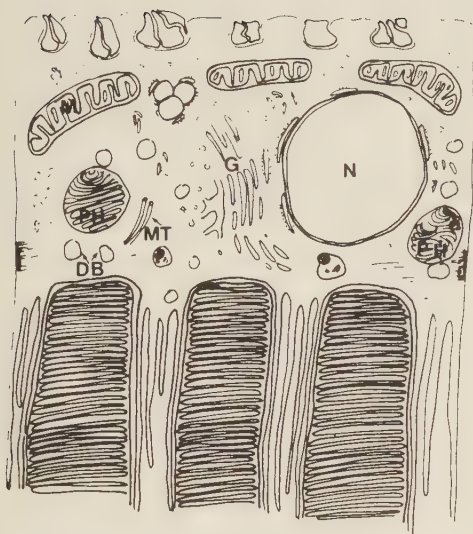
- a) A control cell 4 to 8 hours after a 0.9 % NaCl administration. Dense bodies (DB) can be seen to be randomly scattered throughout the cell often being adjacent to, or in direct contact with a phagosome (PH). Microtubules (MT) can be found in the cytoplasm.
- b) A RPE 4 to 8 hours after a 0.5 mg colchicine administration. Notable dense bodies (DB) which are clumped together, can be seen adjacent to the Golgi apparatus (G) and GERL (GL). Crystalloid structures (arrows) can be identified in some of the dense bodies. Many multivesicular bodies (MV), as well as large autophagic vacuoles (AV), can be seen. Mitochondria (M) are plentiful and often are in a state of autophagy (double arrow). Centrioles (C) can sometimes be identified with a 9 + 0 arrangement. No dense bodies are seen closely associated to the phagosome (PH). An increase in ER, especially of the smooth type (SER) is often found after colchicine treatment.

c) and d) : Portion of the retina incubated in a modified Gömöri medium in order to display sites of acPase activity. The choriocapillaris (CH), the RPE, and the outer and inner segments of the rod cells (ROS, RIS), can be identified in these 2 diagrams.

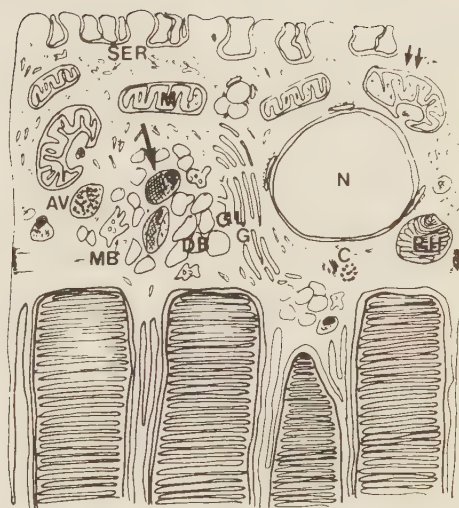
- c) Four hours after a 0.9 % NaCl administration. AcPase activity is seen as an evenly dispersed, finely granular deposit (arrows) on the RPE. No reaction product can be seen on the other retinal layers.

d) Four hours after a 0.5 mg colchicine administration. A heavy clumping of lead sulphate precipitation can be observed in the RPE (arrows). No reaction product is seen in the other retinal layers.

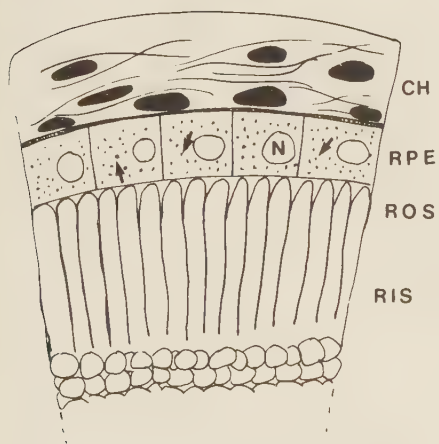




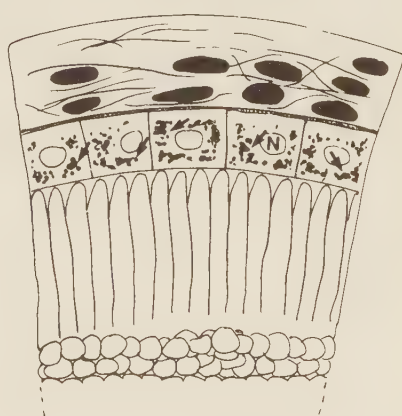
a.



b.



c.



d.

## D I S C U S S I O N

The morphological and functional changes occurring during the development of the retina in the albino rat have been the object of only a few studies in the past (Braekevelt and Hollenberg, 1970; Dowling and Gibbons, 1962; Weidman and Kuwabara, 1968, 1969).

While carrying out this present study, emphasis was put on a more detailed description of the cytogenesis of different cell organelles, permitting a better understanding of some of the cell organelle interrelationships in the RPE, in particular the phagolysosomal system.

Notable changes were observed in various cell organelles of the RPE throughout development. The ER, especially of the smooth type (SER) which showed acPase activity, was seen to be associated with the maturation of the Golgi apparatus and with the formation of lysosomes and premelanosomes. This was observed not only in this study, but in others (Boutry and Novikoff, 1975; Novikoff, 1967; Maul, 1969; Stanka, 1971). AcPase activity was seen in a specialized part of the SER closely related to the Golgi apparatus and referred to as GERL (Golgi-Endoplasmic-Reticulum-Lysosome) by Novikoff in 1964.

An interesting phenomena observed while carrying out this study was the presence and eventual disappearance of the premelanosomes. Initially seen to develop from acPase-positive SER tubules, they then went through different developmental stages and readily displayed acPase activity. This enzymatic activity might play a role in regulating the growth of the granule. As degeneration of the granule began to take place, the enzyme activity disappeared until the final stages when fusions of premelanosomes with acPase-

positive dense bodies were observed, the end product being a residual body.

Phagosomes, first seen in the SIV-50 rat's RPE cytoplasm by 2 weeks of age marks the onset of the process of phagocytosis of the continually shedded ROS. The engulfment of the ROS tips and their progressive break-down in the RPE cytoplasm, were seen to be paralleled by a contact and fusion with dense bodies, a phenomenon similar to that seen during the premelanosomal degradation. Previous to the phagolysosomal contact most of the lysosomes were seen to be freely dispersed in the cytoplasm. Phagocytosis continues throughout the RPE cell's life. Since the rate of mitosis is very low, residual bodies, mostly lipofuscin granules, increase in number during ageing. The observed increase in acPase activity in older cells, which varied considerably from cell to cell, is thought to be the result not only of increased latency of the lysosomal enzymes, known to occur in old age (Tappel, 1968), but also to a leaking of lysosomal membranes commonly observed in old age (Comfort, 1966; Packer et al., 1967; Tappel, 1965, 1968).

The first experimental condition, subjecting rats to varying conditions of illumination produced interesting results in younger animals. Premelanosomal degeneration and the abundance of phagosomes in the RPE cytoplasm were considerably reduced in darkness (table I and II). From these results, it was suggested that prolonged dark adaptation may bring about a slowing down of intracellular movements of premelanosomes towards the apical microvilli and their final fusion with acPase carrying dense bodies. ROS shedding may be a light induced process accounting for the decrease in phagosomes in dark reared animals.

The second experimental condition in which the SIV-50 rat was subjected to colchicine treatment, produced notable changes in cell organelle interactions, in particular a conspicuous accumulation of acPase-positive dense bodies near the Golgi apparatus. Only few or no dense bodies were found in association with phagosomes, which were of a normal appearance. It was concluded that colchicine had an effect on blocking or inhibiting the intracellular pathways which normally allow the dense bodies to come into contact with the material to be degraded. Microtubules have been shown in other cells to be responsible for intracellular transport mechanisms (Bilke et al., 1966; Le Marchand et al., 1973, 1974; Orci et al., 1973; Porter, 1966; Schmitt and Samson, 1968), and colchicine has been shown to bind to the microtubular subunit protein bringing about its dissociation (Borisy and Taylor, 1967a, 1967b; Daniels, 1972; Shelanski and Taylor, 1967, 1968). Since microtubules were seen to disappear in this study on the RPE and in another (Hansson and Sjöstrand, 1972) it was suggested that microtubules are normally responsible for the intracellular mechanisms in the RPE.

S U M M A R Y

A morphological study was carried out on the retinal pigment epithelium (RPE) of the SIV-50 albino rat, a Sprague-Dawley strain, in order to elucidate some aspects of the phagolysosomal system at different developmental stages and under different functional conditions. The animals used for this study varied in age from 16 days of gestation to 120 weeks old (senile). All material examined was prepared for conventional electron microscopic studies as well as for electron and light microscopic cytochemical studies, the latter of which was used to localise sites of acid phosphatase (acPase) activity and glucosaminidase activity.

Two experimental conditions were used in this study, the first subjecting rats to dark rearing, the second, administering, intravenously, colchicine to young rats.

In the RPE from very young, normal animals, changes during development were seen to occur in the endoplasmic reticulum (ER), the Golgi apparatus, mitochondria and premelanosomes. The different developmental stages, as well as the eventual degeneration of premelanosomes, along with the enzymatic activities was studied and discussed in detail. Emphasis was put on the possible role of dense bodies in premelanosomal break-down. The absence of light was seen to slow down this degradation process.

Phagocytosis of shedded rod outer segments (ROS) was first seen to take place by 2 weeks of age in the normal animal and continued throughout the cell's life. The phagolysosomal break-down appeared to be related to a contact and fusion with dense bodies. In older and senile tissue,

large accumulations of residual bodies, mostly lipofuscin granules, were observed, as a result of the continued phagocytosis of the ROS. The absence of light in young animals, showed a slowing down in phagocytosis. From these results, it was suggested that light is implicated in ROS shedding.

Colchicine treatment brought about large, noticeable Golgi-oriented dense body aggregates, which increased in size proportionally with the reaction time of the drug. Both acPase and glucosaminidase activity was seen on these aggregates especially noticeable as large, darkly stained clumps at a light microscopic level. Even though acPase-positive phagosomes were seen in the colchicine treated cells, dense bodies were rarely seen in their immediate proximity. These results of these experiments are discussed and it is suggested that the intracellular transport mechanisms are inhibited, probably being related to a block of microtubule assembly.

## R E S U M E

Ce travail a pour but l'étude du système phagolyso-somial de l'épithélium pigmentaire rétinien (RPE) du rat albinos SIV-50 (souche Sprague-Dawley).

Les techniques cytochimiques ont permis d'étudier en microscopie électronique et optique les différents stades du développement de l'embryon de 16 jours au rat sénile de 120 semaines.

De plus, des animaux ont été soumis à deux conditions expérimentales : action de l'obscurité et action de la colchicine. Au cours du développement du jeune animal, des modifications notables s'observent au niveau du réticulum endoplasmique, de l'appareil de Golgi, des mitochondries et des prémélanosomes. Les changements morphologiques et enzymatiques observés dans ces organites cellulaires sont décrits et discutés en détail.

Dès la deuxième semaine, des phagosomes, résultant de la phagocytose des segments externes des photorécepteurs, apparaissent dans le cytoplasme des cellules. Leur nombre tend à s'accroître avec l'âge. Les prémélanosomes, associés aux lysosomes subissent une suite de modifications morphologiques et biochimiques qui conduira à leur disparition.

Les relations entre les différentes organites cellulaires impliquées dans la dégradation des prémélanosomes, en particulier le rôle joué par les lysosomes dans la dégradation, est décrit et discuté en détail.

L'adaptation à l'obscurité inhibe la phagocytose des segments externes et retarde la dégradation des prémélanosomes. Ces faits indiqueraient que la phagocytose des segments



externes et la disparition des prémélanosomes chez le rat albinos est induite par la lumière.

Le traitement à la colchicine provoque d'impressionnantes agrégations des granules denses, surtout dans la région de l'appareil de Golgi. Ces agrégations sont d'autant plus importantes que l'action de la colchicine dure, et ceci est aussi observé avec leurs activités enzymatiques. Les phagosomes des cellules traitées montrent une activité acide phosphatasique, mais contrairement aux cellules de contrôle, il n'y a que rarement des lysosomes à leur proximité.

Ces résultats suggèrent que les mécanismes de transport intracellulaire d'hydrolases acides sont, soit bloqués, soit sévèrement inhibés par la désorganisation des microtubules.

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KEY TO ABBREVIATIONS

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|     |                                |
|-----|--------------------------------|
| AV  | : Autophagic vacuoles          |
| BI  | : Basal infoldings             |
| C   | : Centriole                    |
| CH  | : Choriocapillaris             |
| CJ  | : Cell junction                |
| CV  | : Coated vesicle               |
| DB  | : Dense body                   |
| ER  | : Endoplasmic reticulum        |
| G   | : Golgi apparatus              |
| GL  | : GERL                         |
| L   | : Lipid droplet                |
| M   | : Mitochondrion                |
| MB  | : Multivesicular body          |
| MF  | : Microfibrils                 |
| MT  | : Microtubule                  |
| MV  | : Apical microvilli            |
| N   | : Nucleus                      |
| P   | : Premelanosome                |
| PH  | : Phagosome                    |
| R   | : Ribosome                     |
| RB  | : Residual body                |
| RER | : Rough endoplasmic            |
| RIS | : Rod inner segments           |
| ROS | : Rod outer segments           |
| RPE | : Retinal pigment epithelium   |
| SER | : Smooth endoplasmic reticulum |
| TF  | : Tonofibrils                  |









